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Genetic diversity among populations in Scotch pine (*Pinus silvestris* L.) seed stands of Western Black Sea Region in Turkey

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The aim of this study was to determine the differences among some of the registered seed stands in the Western Black Sea Region in Turkey by means of the morphological characters. The number of 18 morphological characters were designated by measuring and observing on seed (seed length, seed width, seed color and 1000 seed weight), juvenile seedling (root length, hypocothyl length, epycotyl length, cotyledon number, cotyledon length, stem and root dry weight) and seedling (root-collar diameter, number of branches, bud number, stem length, root length, stem and root dry weight) from 9 seed stands in the Western Black Sea Region of Turkey. The obtained data was analyzed by using cluster and penrose analysis. Cluster and penrose analysis showed that there were significant differences within the *Pinus sylvestris* stands for the morphologic characters.

Key words: Genetic diversity, *Pinus sylvestris,* Scotch pine, morphological characters.

INTRODUCTION

Genetic variation is the fundamental component, which ensures survival and thus, the stability of forest ecosystems as its quantity and quality, determines the potential of population to adapt the changing in environmental condition. This is particularly important with changing population and climatic condition and when the long-term stability of forest ecosystems is increasingly threatened by environmental stress. Thus, a genetic characterization of natural forest resources is the first step necessary for a better understanding of genetic resources for imple-mentation of *in situ* and *ex situ* conservation activities (Turna et al., 2006).

Scots pine (*Pinus sylvestris* L.) is the most widely distributed member of the family *Pinaceae* in the world. The longitudinal range of Scots pine covers over 14,000 km and extends from 8°W in Spain to 141°E in Siberia.

The latitudinal range of Scots pine covers over 3,700 km and extends from 37°N in Turkey to 70°N in Norway, Sweden and Finland (Turna, 2003; Critchfield and Little, 1966). The species covers a diverse array of physiographic regions. Throughout much of its native range in Turkey, Scots pine is an important commercial forest tree species, widely planted for industrial and conservation activities such as protecting soil erosion and living snowfences. Scots pine occupies about 738,000 ha in Turkey, growing mainly in the Black Sea coastal mountains on warm southern slopes, where the climate is humid. In the inner mountains further south, which are also parallel on the Black Sea coast, the tree species grows primarily on humid northern slopes. The southern boundary of its geographical distribution in Turkey lies at around 38° 34' N latitude. On the eastern coast of the Black Sea, it grows at altitudes of 2000 – 2700 m. On the eastern region, it ranges from 1200 to 1600 m. In exceptional cases, it is distributed vertically from sea level (Trabzon-Çamburnu) up to the Alpine Zone (Zigana Mountain) as high as 2400 m in Northeastern Black Sea Region. Within its distribution

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range, Scots pine can grow under diverse ecological conditions. Such a wide range of ecological conditions could favor the formation of a variety of ecotypes (Turna, 2003; Turna and Guney, 2009).

Scotch pine is an important component of subalpine ecosystems of Black Sea Region in Turkey because of its role in watershed protection, its functional natural habitat for wild life, also, because of its esthetic values. It has, therefore, great importance especially in Black Sea forestry. Scotch pine is one of the important forest tree species both Turkish Forestry and breeding program. Although it has 21 seed orchard in 109.4 ha and 36 seed stands 4878.3 ha, 91% of seed demand is covered from seed stands for afforestation in the species (Ayan et al., 2005).

Seed stand is the most important seed source in the species at this stage. Determination of similarity/differences among population/seed stands is an important stage of breeding program. It should known to obtain maximum variability among genotypes and minimum loss of genetic diversity in current generation. It is also important for sustainable forestry and to transmit the current gene diversity to the next generation by afforestation with suitable seed source. Besides, it can be used to the determination of breeding population and gene conservation areas and for establishment of seed orchards in the species. Morphological distance can play important role for the purposes.

In this study were to investigate the morphological variation among Scotch pine populations in Western Black Sea Region of Turkey, using nine different populations. Some populations of Scotch pine in Western Black Sea Region were grouped to contribute breeding, afforestation, artificial regeneration, gene conservation and nursery practice of the species.

MATERIALS AND METHODS

Seed collection and sowing

Open pollinated seed materials from nine different populations of *P. sylvestris*, collected from Western Black Sea Region, were provided by Forest Tree Seed and Tree Breeding Research Directorate in Turkey. Bulked seeds for each population were collected from already established stands whose areas range from 7.5 ha to 150 ha. Locations and description of the studied population are indicated in Figure 1 and Table 1. In this study, Scotch pine seeds were shown two different conditions. One of them was in a greenhouse and the other was open nursery condition. While some seeds were sown at seed beds at 0.5 cm depth 10 cm x 1 cm spacing (250 seeds m^2) with 5 replications in a greenhouse (for cotyledon number and length, epycotyl and hypocotyls length, stem-root dry weight and root length). Seeds were sown to river sand, forest soil and peat in 1:1:2 proportion, respectively, and covered by perlite, the other seeds were sown by using conventional methods at Forest Nursery in Taşköprü-Kastamonu (Altitude: 1160 m) with 6 replications (for stem-root length, root-collar diameter, number of branches, bud number and stem-root dry weight). The

seed density was 500 m^{-2} in the nursery conditions.

Seed and seedling morphological variables studied and data collection

Seed length and width were measured from 200 seeds, 1000 seeds weight were measured from ISTA and proportion of white colored seeds from 800 seeds. While cotyledon number and length, epycotyl and hypocotyls length, stem-root dry weight and root length were measured from 90 seedlings, chosen randomly, which were 2 months old (and obtained) from the greenhouse, stem-root length, root-collar diameter, number of branches, bud number and stem-root dry weight were measured from 90 seedlings, chosen randomly, which were 1 year old (and obtained) from nursery. The dry weights were determined after samples were oven-dried at 105°C for 24 h.

Statistical analyses

Collected data was standardized before the calculations (Turna et al., 2001) and the seedling morphological distance among populations were estimated as:

$$Z_{i,k} = \left[\frac{X_{i,k} - X_{aver}}{S_k} \right]$$

Where $Z_{i,k}$ is standardized values of the kth characteristics of the Ith population, $X_{i,k}$ is original average of the kth characteristics of the Ith populations for the kth characteristics and S_k is the standard deviation of the studied populations for the kth characteristics.

$$D_{i,j} = \sum_{k=1}^{n} \frac{\left[\mu_{k,i} - \mu_{k,j} \right]^2}{n V_k}$$

Where, D_{ij} is the morphological distance between the ith, population and the jth populations, n is the number of studied characteristics, μ_{kj} is the standardized values of the kth of the Ith population, μ_{kj} is the standardized values of the kth characteristics of the jth population and V_k is the variance of standardized averages of the kth characteristics (Ayan et al., 2005; Yahyaoğlu et al., 2001).

RESULTS AND DISCUSSION

The mean values and standard deviation of seed charac-ters by populations are shown in Table 2. Population of Ilgaz Yenice (P1) for seed length, population of Daday Ballıdağ (P6) for seed width, population of Daday Sarıçam (P7) for seed collar and population of Beypazarı Eğriova for 1000 seed weight showed highest perfor-mance. Population of Bolu Aladağ (P5) for seed length, population of Eskipazar Ulupınar (P2) for seed width, population of Akyazı Dokurcun (P4) for seed collar and population of Daday Sarıçam for 1000 seed weight showed lowest performance. Average seed length is 4.86 mm, seed width is 2.85 mm, seed collar is 34.03 % and 1000 seed weight is 10.08 g. Turna (2003) investigated variation of 11 Scotch pine population in Turkey from 900
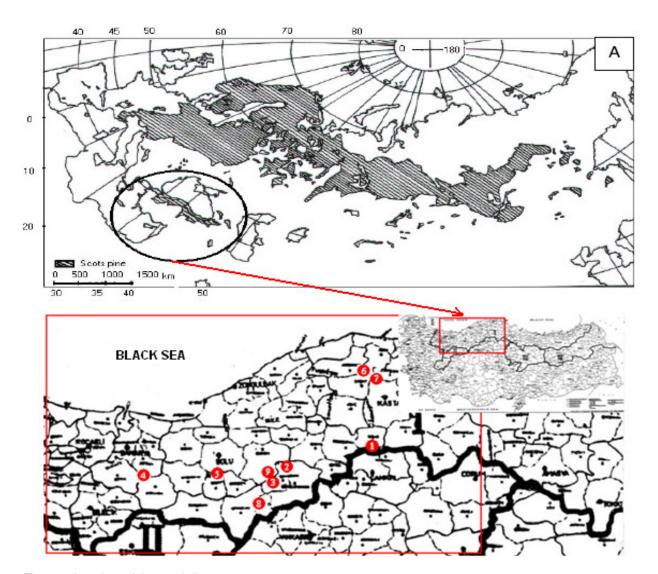
Figure 1. Locations of the populations.

to 2350 m altitude and this investigation showed that average seed length is 4.71 mm and seed width is 2.66 mm.

The mean values and standard deviation of juvenile seedlings characters by populations are shown in Table 3. Population of Ankara-Eğriova (P8) showed the highest performance for all juvenile seedling characters except root length. For root length population of Çamlıdere Benliyayla2 showed highest performance. Population of Bolu Aladağ (P5) for root length and hypocothyl length, population of Daday Sarıçam (P7) for cotyledon length and stem dry weight, population of Eskipazar Ulupınar (P2) for cotyledon number and root dry weight and population of Ilgaz Yenice for epycotyl length showed lowest performance. Average root length is 75.26 mm, hypo-cothyl length is 15.9 mm, epycotyl length 13.44 mm, cotyledon number is 6.64 cotyledon lengths is 24.31 mm,

stem dry weight is 16.5 g and root dry weight is 7.37 g.

The mean values and standard deviation of mature seedling characters by populations are shown in Table 4. Population of Akyazı Dokurcun (P4) showed the highest performance for root-collar diameter, number of branches, stem and root dry weight. Population of Akyazı Dokurcun (P4) showed the lowest performance for bud number. Population of Beypazarı Eğriova (P8) showed highest performance for stems and root length. Population of Bolu Aladağ (P5) showed highest performance for bud number. Population of Ilgaz Yenice (P1) showed the lowest performance for number of branches, stem length and stem dry weight. Population of Daday Ballıdağ (P6) showed the lowest performance for root-collar diameter and root dry weight. Population of Eskipazar Ulupınar (P2) showed the lowest performance for root length. Average root-collar diameter is 1.52 mm, number of

Table 1. Description of the studied populations in Turkey.

Pop. No	Population names	Altitude (m)	Latitude (N)	Longitude (E)	Aspect
1	ANKARA-Ilgaz-Yenice	1500	41° 02' 40''	33° 47' 36''	NW
2	ANKARA-Eskipazar-Ulupınar	1550	40°53' 25''	32° 20' 20''	SS
3	ANKARA-Çamlıdere-Benliyayla 1	1550	40° 31' 40''	32° 08' 00''	SE
4	ADAPAZARI-Akyazı-Dokurcun	1400	40° 37' 30''	30° 50' 00''	S
5	BOLU-Aladağ-Aladağ	1400	40° 38' 00''	31° 41' 30''	W
6	KASTAMONU-Daday-Ballıdağ	1300	41° 34' 00''	33° 19' 50''	NW
7	KASTAMONU-Daday-Sarıçam	1250	41° 22' 18''	33° 28' 54''	Various
8	ANKARA-Beypazarı-Eğriova	1550	40° 26' 30''	32° 02' 03''	Various
9	ANKARA-Çamlıdere-Benliyayla 2	1550	40° 31' 40''	32° 08' 00''	N

Pop. No. = Population number.

Table 2. Averages of the studied seed characteristics.

Pop. code	Seed length (mm)		*Seed width (mm)*		Seed color (White seed %)		1000 seed weight (g)	
1	4.96	± 0.44	2.84	± 0.25	35.88	± 3.27	10.19	± 3.27
2	4.84	± 0.47	2.80	± 0.26	29.13	± 5.49	10.40	± 3.29
3	4.77	± 0.48	2.81	± 0.26	37.38	± 5.04	10.35	± 3.32
4	4.90	± 0.51	2.90	± 0.27	27.75	± 6.65	10.28	± 3.30
5	4.73	± 0.49	2.82	± 0.27	34.63	± 4.84	9.78	± 3.14
6	4.89	± 0.45	2.92	± 0.26	35.88	± 5.28	10.44	± 3.35
7	4.88	± 0.50	2.85	± 0.25	42.00	± 3.63	8.77	± 2.81
8	4.92	± 0.51	2.86	± 0.27	32.75	± 4.27	10.54	± 3.38
9	4.82	± 0.52	2.83	± 0.25	30.88	± 4.97	9.97	± 3.20
Average	4.86	± 0.49	2.85	± 0.26	34.03	± 4.83	10.08	± 3.23

Pop. = Population.

Table 3. Averages of the studied juvenile (or 2 month old) seedling characteristics.

Pop. code	Root length (mm) ($\overline{x}$ ± S)	Hypocothyl length (mm) ($\overline{x}$ ± S)	Epycotyl length (mm) ($\overline{x}$ ± S)	Cotyledon number ($\overline{x}$ ± S)	Cotyledon length (mm) ($\overline{x}$ ± S)	Stem dry weight (mg) ($\overline{x}$ ± S)	Root dry weight (mg) ($\overline{x}$ ± S)
1	70.25 ± 21.83	15.00 ± 2.07	11.85 ± 3.70	6.40 ± 1.12	23.29 ± 3.40	14.25 ± 4.70	6.32 ± 2.60
2	68.73 ± 21.15	15.16 ± 2.02	12.73 ± 3.99	6.18 ± 1.10	23.19 ± 2.86	14.88 ± 5.08	5.96 ± 2.63
3	76.53 ± 19.84	15.90 ± 2.53	13.37 ± 3.51	6.55 ± 0.78	24.59 ± 3.62	16.24 ± 4.72	7.73 ± 3.10
4	80.48 ± 16.47	16.73 ± 2.06	13.81 ± 3.43	6.64 ± 0.81	25.37 ± 3.89	17.32 ± 4.86	8.09 ± 2.77
5	68.56 ± 19.73	14.61 ± 2.09	12.79 ± 3.58	680 ± 0.90	24.53 ± 3.66	15.50 ± 4.64	7.05 ± 2.79
6	80.93 ± 18.44	15.81 ± 2.56	14.16 ± 3.29	6.80 ± 0.79	24.57 ± 3.70	16.97 ± 4.59	7.58 ± 2.69
7	71.47 ± 21.95	15.08 ± 2.15	12.09 ± 3.93	6.57 ± 0.72	22.38 ± 2.65	14.24 ± 3.88	6.27 ± 2.24
8	79.39 ± 18.59	18.02 ± 2.38	15.31 ± 3.74	7.02 ± 0.85	25.68 ± 3.61	19.91 ± 4.82	8.94 ± 2.89
9	81.08 ± 18.56	16.76 ± 2.79	14.97 ± 4,20	6.79 ± 0.86	25.26 ± 4.35	19.17 ± 6.70	8.32 ± 3.74
Average	75.26 ± 19.62	15.9 ± 2.29	13.44 ± 3.71	6.64 ± 0.88	24.31 ± 3.53	16.5 ± 4.88	7.37 ± 2.83

Pop. = Population.

branches 1.61 bud number is 1.26 stem length is 36.7 mm, root length is 14.86 cm, stem dry weight is 205.83 mg and root dry weight is 173.3 mg.

Morphological distances, obtained from Penrose's

Table 4. Averages of the studied 1 year old seedling characteristics.

Pop. code	Root-collar diameter (mm) ($\overline{x} \pm S$)	Number of branches ($\overline{x} \pm S$)	Bud number ($\overline{x} \pm S$)	Stem length (mm) ($\overline{x} \pm S$)	Root length (cm) ($\overline{x} \pm S$)	Stem dry weight (mg) ($\overline{x} \pm S$)	Root dry weight (mg) ($\overline{x} \pm S$)
1	1.39 ± 0.35	1.26 ± 1.17	1.14 ± 1.08	32.65 ± 7.33	14.94 ± 0.45	164.31 ± 119.79	147.59 ± 84.84
2	1.47 ± 0.36	1.42 ± 1.26	1.14 ± 1.03	32.82 ± 7.68	14.72 ± 1.02	189.19 ± 126.87	182.05 ± 99.40
3	1.55 ± 0.37	1.56 ± 1.23	1.20 ± 1.12	39.25 ± 7.52	15.00 ± 0.00	212.93 ± 135.47	172.28 ± 84.92
4	1.63 ± 0.35	2.16 ± 1.25	1.10 ± 1.08	37.50 ± 7.87	14.87 ± 0.74	256.60 ± 158.89	206.17 ±109.82
5	1.61 ± 0.34	1.76 ± 1.18	1.50 ± 1.06	38.42 ± 7.84	14.97 ± 0.14	217.69 ± 119.70	178.01 ± 75.44
6	1.37 ± 0.28	1.52 ± 1.13	1.18 ± 1.08	33.83 ± 7.07	14.86 ± 0.70	167.91 ± 86.47	146.34 ± 58.66
7	1.52 ± 0.32	1.53 ± 1.21	1.32 ± 1.22	34.80 ± 6.93	14.81 ± 0.83	225.13 ± 150.78	186.76 ± 93.86
8	1.59 ± 0.27	1.51 ± 1.16	1.48 ± 1.16	41.82 ± 8.48	15.00 ± 0.00	212.98 ± 96.87	173.33 ± 61.83
9	1.52 ± 0.33	1.71 ± 1.30	1.32 ± 1.02	39.17 ± 9.14	14.89 ± 0.64	206.28 ± 134.36	167.51 ± 75.45
Average	1.52 ± 0.33	1.61 ± 1.21	1.26 ± 1.09	36.70 ± 7.76	14.89 ± 0.51	205.83 ± 125.47	173.30 ± 82.70

Pop. = Population.

Table 5. Morphological distance among populations.

Pop. No.	1	2	3	4	5	6	7	8
1	---							
2	1.6235	---						
3	2.2886	2.0111	---					
4	1.4830	2.3297	2.4803	---				
5	1.3682	1.9315	1.5647	1.9547	---			
6	1.4655	1.8465	1.5142	1.2916	1.5059	---		
7	2.2292	1.9122	2.3031	2.5220	3.0068	2.8973	---	
8	1.3949	1.4369	3.1573	3.8252	2.0420	3.4171	2.3249	---
9	1.2622	1.4024	1.6811	2.2512	1.5499	1.8148	1.3545	1.5365

Pop. No. = Population number.

equation, can play important role for the purpose. The different characteristics that have different units are pooled while calculations in morphological distances, obtained the Penrose equation, approach to zero, the similarity among populations increases. Calculated morphological distance among populations are shown that Table 5. The morphological distance value varied from $P_{1,9}$ = 1.2622 (Ilgaz-Yenice; Çamlıdere-Benliyayla2) to $P_{4,8}$ = 3.8252 (Daday-Ballıdağ; Beypazarı-Eğriova). The highest morphological distance values are 3.8252 ($P_{4,8}$); 3,4171 ($P_{6,8}$) and 3.1573 ($P_{3,8}$), the lowest morphological distance values are 1.2622 ($P_{1,9}$); 1,2916 ($P_{4,6}$) and 1.3545 ($P_{7,9}$).

Seed and seedling morphology are a more recent approach, where very early developmental stages show appropriate stability. The advantage of using seedling morphology stems from their uniformity at the juvenile stages, before they are subjected to the diversity of factors that prevail in the case of mature plants.

It could be explained that large genetic diversity and geographic variation among populations even they were from the same district. The similar results were found by Yahyaoğlu et al. (2001) on *Cedrus libani* A rich. Classifications of genotypes were studied detail by different distances by Crossa and Franco (2004).

Hierarchical Cluster analysis was applied by standardized values in SPSS statistical package program. Dendogram of hierarchical cluster analysis, based on morphological characters is shown in Figure 2.

Morphological distance and grouping

Populations of Ilgaz-Yenice and Ankara-Benliyayla2 were the most similar ($D_{P1, P9}$ = 1.2622), populations of Akyazı-Dokurcun and Beypazarı-Eğriova ($D_{P4, P8}$ = 3.8252) were the most different to each other (Table 5). Such a high D values might be the result from varying local ecological
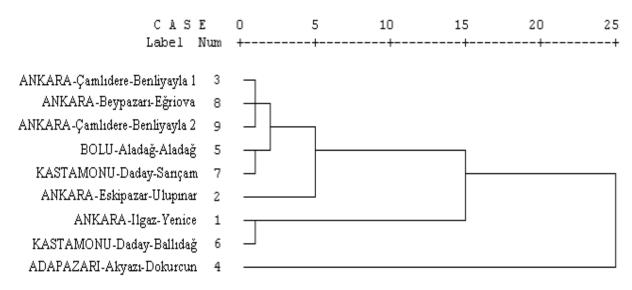
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C A S E       0       5      10      15      20      25
Label  Num  +---------+---------+---------+---------+---------+
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ANKARA-Çamlıdere-Benliyayla 1   3
ANKARA-Beypazarı-Eğriova   8
ANKARA-Çamlıdere-Benliyayla 2   9
BOLU-Aladağ-Aladağ   5
KASTAMONU-Daday-Sarıçam   7
ANKARA-Eskipazar-Ulupınar   2
ANKARA-Ilgaz-Yenice   1
KASTAMONU-Daday-Ballıdağ   6
ADAPAZARI-Akyazı-Dokurcun   4

Figure 2. Dendogram of hierarchical cluster analysis.

conditions. Although populations of Ankara-Benliyayla (P3 and P9, $D_{P3, P9}$ = 1.6811) were from the same district, Ankara-Benliyayla2 population was the most similar to Ilgaz-Yenice population ($D_{P1, P9}$ = 1.2622). Turna reported that it is hard to see apparent relations and their geographic locations. For example, while Sarıkamış and Şenkaya are geographically close to each other but their genetic distance is far apart from each other. This result showed that seeds from Ilgaz-Yenice population could be used as a seed source instead of Ankara-Benliyayla2 for the similar afforestation area, when enough seeds were not collected from Ankara-Benliyayla2 population.

Similarities among populations were shown in Figure 2 by Hierarchical cluster analysis. According to results of the cluster analysis, Ankara-Yenice (P1), Kastamonu-Ballıdağ (P6) and Adapazarı-Dokurcun (P4) population were in the main same group and the other populations were in another main same group. When seen sub-group it was evident that Adapazarı-Dokurcun (P4) population was very different than the others (Figure 2). It could be because of its longitude and different ecological and genetical material condition (Table 1). Results of the cluster analysis (Figure 2) were well accordance with morphological distances (Table 5). For instance, morphological distance of Ankara-Benliyayla1 (P3), Ankara-Eğriova (P8) and Ankara-Benliyayla2 (P9) populations to Adapazarı-Dokurcun (P4) were the highest (D $_{P3, P4}$ = 2.4803 , D $_{P8, P4}$ = 3.8252 , D $_{P9, P4}$ = 2.2512) than the others. It can be suggested that all populations, especially Ankara-Banliyayla1 (P3) and Adapazarı-Dokurcun (P4) populations, be considered for a gene conservation program. Also, future studies are necessary to provide deeper insights in to the subject.

These results could be used in preparation of gene

map, seed transfer zones, determination of breeding populations, gene conservation areas, geographic variation and resulting of provenance trials of the species in short period. Preparation of forest gene maps and determination of seed transfer zones and geographical variation by morphological distance were also suggested by Yahyaoğlu et al. (2001).

It may be concluded from the present study that studied characteristic were the important factors on morphological distance among populations and grouping populations. There was a large genetic and geographic variation among the population; they even were at the adjacent district. Morphological distance among population could be taken into consideration in silvicultural purpose (afforestation, artificial regeneration) and breeding strategies (that is, determination of breeding populations, gene conservation areas, seed transfer zones, seed sources and geographic variation, resulting of provenance trial; establishment of seed orchard) of this species.

Generally, our results show that large genetic diversity exist in *P. sylvestris* to explain its great ecological plasticity and evolutionary. The results of this study showed that the populations are not homogeneous with regard to the characteristic of seed, juvenile seedling and seedling. Populations consist of the trees having more or less different characteristics in seed, juvenile seedling and seedling. The reason of the fact that the grouping and differences existed among the studied population in terms of the morphological characters may explain that there were different origins or varieties forming the Scot pine stands. Variation in most of these characteristics appeared to be related to altitude, divergent gene and genotype frequencies. The result is similar to the findings of Chmura (2006) for *Picea abies* and Kara et al. (1997),

Isık and Kara (1997), Isık (1986) for *Pinus brutia.* The result reported here for *P. sylvestris* are in accordance with the findings of Alia et al. (2001), Harju et al. (1996), Nilsson and Walfridsson (1995), Prus-Glowacki and Stephan (1994), Hertel and Kohlstock (1994) Ayan et al. (2005) and Bilgen and Kaya (2007).

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